Journal of Ethnopharmacology 137 (2011) 1143–1148

View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Preclinical evaluation of *Trichilia catigua* extracts on the central nervous system of mice

Janaine M. Chassot[a], Renata Longhini[b], Lucas Gazarini[a], João Carlos P. Mello[b], Rúbia Maria Weffort de Oliveira[a,*]

[a] *Laboratory of Neuropsychopharmacology, Department of Pharmacy and Pharmacology, State University of Maringá, Av. Colombo n. 5790, 87020-900 Maringá, Paraná, Brazil*
[b] *Laboratory of Pharmacognosy, Department of Pharmacy and Pharmacology, State University of Maringá, Av. Colombo n. 5790, 87020-900 Maringá, Paraná, Brazil*

ARTICLE INFO

Article history:
Received 1 March 2011
Received in revised form 4 July 2011
Accepted 10 July 2011
Available online 20 July 2011

Keywords:
Trichilia catigua Juss (Meliaceae)
Antidepressant
Memory
Total phenolics
Total tannins
DPPH

ABSTRACT

Ethnopharmacological relevance: Trichilia catigua preparations have been popularly used in Brazil as a tonic for the treatment of fatigue, stress, impotence, and deficiency of memory. The aim of the present study was to investigate the possible antidepressant, anxiolytic, motor and cognitive effects of the crude extract (CE) or ethyl-acetate fraction (EAF) of *Trichilia catigua*. Analyses of the total phenolics and total tannins content, as well as the *in vitro* antioxidant activity of CE and EAF were also performed.
Materials and methods: CE (200–800 mg/kg) and EAF (100–400 mg/kg) were orally administered to mice and 1 h later the behavioral tests were performed. The free radical scavenging activity was measured by using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) method.
Results: Single administration of CE (200–400 mg/kg) or EAF (100–400 mg/kg) did not change the behavior of the animals submitted to the elevated plus maze or their locomotor activity in the open field test. An antidepressant-like effect was detected with EAF (400 mg/kg) after acute administration. Both CE (800 mg/kg) and EAF (200 and 400 mg/kg), improve memory in mice as measured by an increased latency in the step-down inhibitory avoidance test. The EAF presented higher total phenolics and total tannins as compared to CE as well as it exhibited the best antioxidant activity.
Conclusions: The present results showed an *in vitro* antioxidant activity for EAF and suggested that it may be useful for cognitive improvement. It is possible that both functional and chemical activities are related.

© 2011 Elsevier Ireland Ltd. Open access under the Elsevier OA license.

1. Introduction

Trichilia catigua Juss (Meliaceae) is a medium-sized flowering tree distributed in some countries of South American. In Brazil, it is known as “catuaba”, “catuama”, or “catiguá”, and has been used in folk medicine as a tonic for the treatment of fatigue, stress, impotence and memory deficits (Pizzolatti et al., 2002). An adaptogen function has been also attributed to *Trichilia catigua*, since it has been used to decrease the consequences of stress and improve physical and cognitive performances both in healthy and ill patients (Mendes and Carlini, 2007).

Some of popular uses of *Trichilia catigua* have been confirmed by experimental approaches. Commercially available preparations containing *Trichilia catigua* have been shown to display relaxant actions in *Corpus cavernosum* strips from rabbits (Antunes et al., 2001), and to present antinociceptive (Viana et al., 2009) and anti-inflammatory (Quintão et al., 2008) properties. In 2005, Campos et al. have demonstrated antidepressant-like effects for *Trichilia catigua* hydroethanolic extract in rodents submitted to the forced swimming test (FST).

The mechanism of action of *Trichilia catigua* preparations are yet unknown. It has been suggested that nitric oxide (NO) and dopaminergic transmissions may be involved in pharmacological properties of *Trichilia catigua* preparations (Calixto and Cabrini, 1997; Campos et al., 2005; Quintão et al., 2008). In addition, some of biological activities of *Trichilia catigua* may derive from its capacity to exert protective and/or inhibitory actions against free radicals (Davydov and Krikorian, 2000). A strong antioxidant activity was described for the constituents of the methanolic extract, acetonic extract, and ethyl-acetate fraction (EAF) of the barks of *Trichilia catigua* by the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging test (Beltrame et al., 2006; Brighente et al., 2007; Tang et al., 2007). It is possible that the antioxidant properties of *Trichilia catigua* are related to the presence of phenolic compounds such as flavonoids, tannins and phenylpropanoids (Tang et al., 2007).

Considering the broad pharmacological effects attributed to *Trichilia catigua* preparations, the aim of this study was to further

* Corresponding author. Tel.: +55 44 30114814; fax: +55 44 30114999.
E-mail address: rmmwoliveira@uem.br (R.M.W. de Oliveira).

0378-8741 © 2011 Elsevier Ireland Ltd. Open access under the Elsevier OA license.
doi:10.1016/j.jep.2011.07.032

characterize the behavioral effects of *Trichilia catigua* crude extract (CE) and a semipurified fraction ethyl-acetate fraction (EAF) in animal models of anxiety, locomotion and memory in mice. The total phenolics and the total tannins present in CE and EAF, as well as the antioxidant effect of these preparations were also investigated.

2. Materials and methods

2.1. Plant material and extracts preparation

The barks of *Trichilia catigua* were collected in São Miguel do Oeste, Santa Catarina, Brazil (2008) and a voucher specimen was identified by Dr. Gerdt Hatschbach and deposited at the Herbarium of Curitiba Town Hall (no. 306253), Curitiba, Paraná, Brazil.

Air-dried stem barks (450 g) was extracted with 4.5 l of acetone–water (7:3) by turbo-extraction (Ultra-turrax® model UTC115KT; USA) for 15 min at temperature ≤40 °C. Next, the crude extract (CE) was filtered and concentrated in a rotavapor under reduced pressure, and lyophilized, for a yield of 101 g. The CE (50 g) was dissolved in water (0.5 l) and partitioned with ethyl acetate, obtaining 13 g of ethyl-acetate fraction (EAF).

2.2. Determination of total phenolic and total tannin contents

The total phenolic content was determined according to the European Pharmacopoeia spectrophotometric method (European Pharmacopoeia, 2007; Verza et al., 2007). Stock solution was prepared from lyophilized CE (0.15 g) or EAF (0.03 g) of powered sample mixed with 250 ml of water. Total polyphenols were determined from a 5 ml aliquot of the stock solution diluted to 25 ml. An aliquot of 2 ml of the formerly prepared solution was mixed with 1 ml Folin–Ciocalteu reagent 2 N (Sigma–Aldrich, EUA), 10 ml water and complete the volume (290 g/l) of sodium carbonate solution volumetric flask (25 ml). The adsorption was measured after 30 min at 760 nm (A1) using water for compensation (Shimadzu UV/vis PC-1650, Japan). Polyphenols unadsorbed on hide powder were determined from 10 ml of stock solution by addition of 0.1 g hide powder (Freiberg®, Germany) vigorously mixed at room temperature for 60 min, and filtered. From this filtrate an aliquot of 5 ml was diluted to 25 ml with water. An aliquot of 2 ml of the formerly prepared solution was mixed with 1 ml Folin–Ciocalteu reagent 2 N (Sigma–Aldrich, USA), 10 ml water and complete the volume (290 g/l) of sodium carbonate solution volumetric flask (25 ml). The adsorption was measured after 30 min at 760 nm (*A*2) using water for compensation. Standard pyrogallol solution was prepared from 50 mg in water diluted up to 100 ml. An aliquot of 5 ml was diluted to 100 ml with water. Two milliliters of thus prepared solution was mixed with 1 ml of Folin–Ciocalteu reagent 2 N, 10 ml of water and complete the volume of 25 ml (290 g/l) of sodium carbonate solution. The adsorption at 760 nm (*A*3) was measured 30 min after pyrogallol dissolution. Tests were carried out in triplicate.

The total tannin (TT) content was determined according to the following relationship:

$$\text{TT}\ (\%) = \frac{62.5 \times (A1 - A2) \times m2}{A3 \times m1}$$

where $m1$ is sample mass in grams and $m2$ is pyrogallol mass in grams.

The results are reported as the mean ± standard deviation (SD) of the total phenolics or total tannins, expressed as pyrogallol percentage and as the relative standard deviation percentage (RSD%).

2.3. Radical-scavenging activity (RSA) assay

The free radical scavenging activities of extract and fraction of *Trichilia catigua* were measured by using 2,2-diphenyl-1-picryl-hydrazyl (DPPH; Amarowicz et al., 2004). Solutions of CE, EAF, Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Sigma) and Vitamin C (ascorbic acid, W.P. Co. Ltd., China) at different concentrations (1–10 μg/ml) were dissolved in 3 ml of methanol and then added to a methanolic solution of free radical DPPH (1 mM, 375 μl). The mixture was strongly shaken and maintained at room temperature for 30 min in the darkness. The absorbance of the resulting solution was read spectrophotometrically (Shimadzu UV/vis PC-1650, Japan) at 517 nm against a blank (2 mg of butylated hydroxytoluene-BHT, dissolved in 4 ml of methanol with 500 μl of the free radical DPPH solution added). The capability to scavenge the DPPH radical or to inhibit free radicals was calculated using the following equation:

$$I\% = \frac{(A_b - A_s)}{A_b} \times 100$$

where *I%* is the capability to scavenge the DPPH radical or to inhibit free radicals, A_b is the absorbance of the control reaction (containing all reagents except the test compounds), and A_s is the absorbance of the test compound. The sample concentration providing 50% inhibition (IC_{50}), concentration required to inhibit DPPH radical formation by 50%, was calculated from the graph of *I%* against sample concentration. Tests were carried out in triplicate. Vitamin C and Trolox® were used as positive control or standard. Data are presented as IC_{50} (μg/ml).

2.4. Animals

Male albino-Swiss mice (30–45 g), housed in groups ($n = 5$) with free access to food and water were used. The experimental procedures adhered to the ethical principles of the Brazilian College of Animal Experimentation (COBEA) and were approved by the Ethics Committee on Animal Experimentation of the State University of Maringá, Paraná, Brazil (Protocol No. 042/2007).

2.5. LD_{50} experiment

Groups of six animals were orally administered with single doses of CE (up to 5000 mg/kg) or EAF (up to 3000 mg/kg). The mice were observed for their gross behavioral, neurologic, autonomic and toxic effects at 15, 30 and 60 min, and at each 2 h short intervals of time up to 48 h and then once a day for 7 days.

2.6. Treatments

Before receiving the treatments, the animals were fasted for 12 h. Saline, CE (200–800 mg/kg), EAF (100–400 mg/kg) or drugs (positive controls) were administered by oral route (gavage) using a tuberculine seringe fitted with oral cannula (0.1 cm × 4 cm). All treatments were administered to mice at a volume of 10 ml/kg.

2.7. Drugs

As controls of behavioral tests, the following drugs were used: Piracetam 200 mg/kg (Nootropil®, Rhodia, SP, Brazil), diazepam 0.2 mg/kg (Dienpax®, Sanofi–Wintrop Laboratories, SP, Brazil) and imipramine hydrochloride 20 mg/kg (Sigma–Aldrich, MO, EUA), which were solubilized in saline (NaCl 0.9%). Diazepam 1 mg/kg was dissolved in vehicle (salina containing Tween-80 2%). The doses were based on previous reports (Lolli et al., 2007; Ergün et al., 2008).

2.8. Behavioral tests

All the behavioral procedures were carried out between 8:00 h and 12:00 h a.m. in a temperature controlled room (23 ± 1 °C),

illuminated with a 40 W fluorescent bulb and were videotaped. A total number of 285 mice (n = 8–16/group) were used for behavioral evaluation. Mice were fasted 12 h prior to drug administration and during the experiments except for repeated treatments. Each animal was used only once, except for those exposed to the open field.

2.8.1. Elevated plus maze

The method was based on Lister (1987). Briefly, the EPM consisted of two open arms (25 cm × 10 cm) and two closed arms (25 cm × 10 cm × 20 cm) that extended from a common central platform (10 cm × 10 cm). The entire maze was elevated to a height of 90 cm above the floor level. The number of open and closed arm entries (CAE), the time spent on open arms and the number of risk assessment (RA) were registered during 5 min for each animal. Subsequently, the percentage of open arm entries (%OAE = 100 × open/total entries) and the percentage of time spent in the open arms (%OT = 100 × open/open + closed time) were calculated.

2.8.2. Open field

Immediately after being tested in the EPM, mice were individually placed in the center of the open field in order to evaluate their locomotor activity (Royce, 1977). The open field used to measure the locomotion was a wooden square box, 45 cm × 45 cm with wall with 30 cm high, which the floor was divided into nine smaller squares of equal dimensions (15 cm × 15 cm). The animals could explore the box during 5 min. Hand operated counters and stopwatches were used to score the number of crossings (number of square floor units entered) and rearing (number of times the animal stood on hind legs).

2.8.3. Forced swim test (FST)

The FST was performed according to the methods described by Porsolt et al. (1977). In brief, mice were individually placed in a 25 cm glass becker (10 cm diameter) containing water at 23 ± 1 °C. Duration of immobility was recorded during a 6-min swimming test. A mouse was judged to be immobile when it floated and its hindlimbs were immobile, and only small movement of the forepaws was made to keep its head above water.

2.8.4. Step-down inhibitory avoidance

The step-down apparatus consisted of an acrylic box (12 cm × 30 cm × 15 cm), whose floor consisted of parallel 1.0 mm diameter stainless steel bars spaced 1.0 cm apart. A 10 cm wide, 3.0 cm high, 6.0 cm long platform occupied the center of the grid floor. In the training session (day 1), immediately after stepping down placing their paws on the grid, the animals received a 0.3 mA 15 s scrambled foot shock and were immediately withdrawn from the cage. Twenty-four hours later, in the text sessions (day 2) no foot shock was given and the step-down latency was used as a measure of retention (to a ceiling of 300 s). Drugs were administered 30 min before the test session, aiming thus to investigate their effect on memory retrieval (Barros et al., 2001). Only animals that showed a latency in the criterion range (30 s) during the training session were used for the retention test.

2.9. Statistical analysis

Data are expressed as mean ± SEM of the groups. Data were analyzed by Student t test or one-way analysis of variance (ANOVA) followed by the Tukey's test for multiple comparisons.

3. Results

3.1. Total phenolics and total tannins

Total phenolics content were 81.12 ± 2.26% (RSD% = 2.78) and 36.63 ± 0.31% (RSD% = 0.84) for EAF and CE, respectively. The total tannins were 54.95 ± 1.32% (RSD% = 2.40) for EAF and 27.27 ± 0.96% (RSD% = 3.52) for CE.

3.2. Radical-scavenging activity (RSA)

ANOVA followed by Tukey's showed significant difference between EAF when compared to CE, Trolox® and Vitamin C ($F_{3,32}$ = 643.99, P < 0.05). The respective IC_{50} (μg/ml) were: CE = 4.51 ± 0.14, EAF = 3.16 ± 0.10, Trolox® = 6.90 ± 0.23, Vitamin C = 4.13 ± 0.08. The EAF exhibited the better antioxidant activity of all compounds. The order of scavenging activity of the samples was as follows: EAF > Vitamin C > CE > Trolox®.

3.3. LD_{50}

The behavior of treated mice appeared normal. No evident toxic effect was observed at oral doses up to 6.25 or 15 times of the effective doses of CE or EAF, respectively. There was no death in any of these groups.

3.4. Elevated plus maze

As expected for a positive control, diazepam (1 mg/kg), induced a selective anxiolytic-like effect in mice characterized by an increase in the %OAE (t_{16} = 5.89, P < 0.05) and of the %OT (t_{16} = 7.23, P < 0.001) spent in the open arms of the EPM compared to vehicle (Table 1). No significant differences were observed on CAE or RA parameters (P > 0.05).

As shown in Table 1, no significant effect was detected with a single administration of CE (200 and 400 mg/kg) or EAF (100, 200 and 400 mg/kg) on EPM anxiety parameters for rats exposed to EPM for 5 min (P > 0.05). Although a statistical trend was detected with CE (800 mg/kg) on open arm exploration parameters (%OAE, $F_{3,39}$ = 2.28, P = 0.10; %OT $F_{3,39}$ = 2.71, P = 0.06) when compared to control group, it did not reach a significant level. There was no statistical difference on CAE and RA parameters when compared to control (P > 0.05).

3.5. Open field

No significant effect was observed in horizontal (crossings) or vertical (rearings) motor activity of mice that received diazepam (1 mg/kg) as compared to those that received vehicle (P > 0.05). Neither CE nor EAF were different of its controls (P > 0.05) (Table 1).

3.6. Forced swimming

The results of FST are demonstrated in Fig. 1. A single administration of imipramine (20 mg/kg) or EAF (200 mg/kg) decreased the immobility time in FST ($F_{7,74}$ = 7.1, P < 0.05), which is characteristic of antidepressant-like effect. However, no effect was detected with EAF (100 and 400 mg/kg) or CE (200–800 mg/kg) administration when compared to control group (P > 0.05).

3.7. Step-down

As observed with piracetam (200 mg/kg), administration of CE (800 mg/kg) or EAF (200 and 400 mg/kg) before the test session

Table 1
Effects of diazepam (DZ; 1 mg/kg), *Trichilia catigua* crude extract (CE; 200–800 mg/kg) and *Trichilia catigua* ethyl-acetate fraction (EAF; 100–400 mg/kg) acute administration in mice ($n = 10$/group) submitted to the elevated plus maze (5 min) and open field (10 min). %OAE = % open arm entries; %OT = % open time; CAE = closed arm entries; RA = risk assessment.

	Elevated plus maze			Open field		
	%OAE	%OT	CAE	RA	Crossings	Rearings
Vehicle	11.0 ± 4.0	8.1 ± 2.2	9.44 ± 0.7	10.67 ± 1.2	55.44 ± 2.7	32.2 ± 2.5
DZ 1 mg/kg	40.6 ± 3.1*	37.5 ± 3.4*	9.67 ± 1.1	9.0 ± 0.9	48.89 ± 2.9	31.4 ± 2.5
Saline	19.13 ± 3.4	5.99 ± 1.5	8.08 ± 1.1	8.92 ± 1.1	61.38 ± 7.2	32.13 ± 3.8
CE						
200 mg/kg	18.19 ± 2.4	7.81 ± 2.2	8.70 ± 1.0	9.90 ± 1.1	64.38 ± 4.3	39.25 ± 4.9
400 mg/kg	13.78 ± 3.4	5.02 ± 1.4	7.00 ± 1.3	9.31 ± 1.6	77.50 ± 2.9	37.38 ± 3.9
800 mg/kg	7.39 ± 4.3+	1.03 ± 0.5+	5.00 ± 1.1	5.62 ± 1.6	65.25 ± 4.24	38.38 ± 2.0
EAF						
100 mg/kg	15.92 ± 2.6	6.81 ± 1.9	8.83 ± 0.8	9.58 ± 1.2	56.50 ± 4.6	33.50 ± 3.9
200 mg/kg	12.87 ± 3.0	4.73 ± 1.3	6.83 ± 1.2	8.75 ± 1.4	61.71 ± 10.4	29.71 ± 6.5
400 mg/kg	10.35 ± 3.4	3.23 ± 1.4	7.31 ± 1.2	7.54 ± 1.2	62.14 ± 9.7	30.71 ± 1.8

* $P < 0.05$ compared to vehicle (Student t test).
\+ $P < 0.10$ compared to saline (ANOVA followed by Tukey's test).

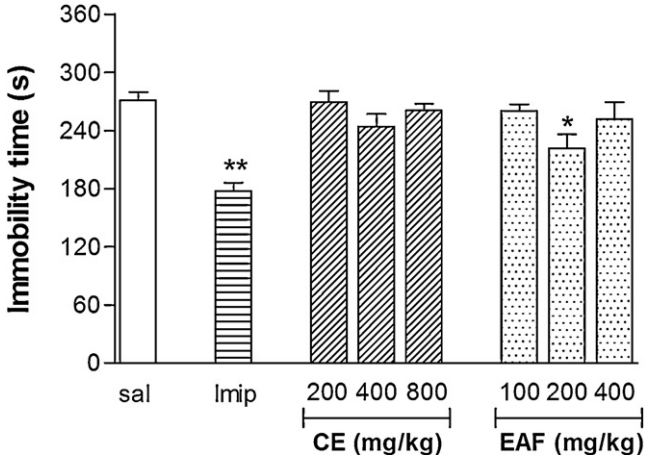

Fig. 1. Antidepressant-like effect of a single oral dose of imipramine (Imip, 20 mg/kg) and *Trichilia catigua* ethyl-acetate fraction (EAF 400 mg/kg) in mice ($n = 8$–10/group) submitted to the forced swimming test for 6 min. *$P < 0.05$, **$P < 0.001$ compared to saline (sal) group (ANOVA followed by Tukey's test).

increased the latency to step-down ($F_{8,121} = 8.62$, $P < 0.05$) as compared to saline group (Fig. 2). Otherwise, diazepam (0.2 mg/kg) significantly reduced the latency time ($P < 0.05$) as compared to saline, indicating significant impairment of memory (Fig. 2). No

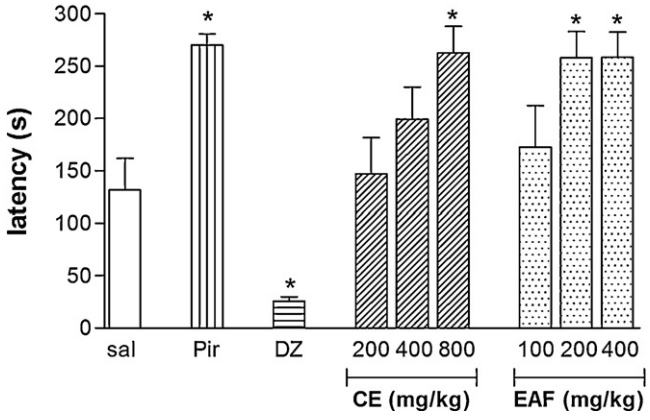

Fig. 2. Effect of *Trichilia catigua* CE (400 mg/kg) and EAF (200 and 400 mg/kg) on inhibitory avoidance evaluated in the step-down test ($n = 8$–16/group). As positive controls, diazepam 0.2 mg/kg (DZ) and piracetam (Pir) were tested. *$P < 0.05$ compared to saline (sal) group (ANOVA followed by Tukey's test).

significant effect was detected in latency to step-down after CE (200 and 400 mg/kg) or EAF (100 mg/kg) administration ($P > 0.05$).

4. Discussion

In the present study, we have investigated the behavioral effects of CE and EAF obtained from the barks of *Trichilia catigua*. No signs of toxicity or deaths were detected 7 days after administration of high doses of CE or EAF. A single oral administration of CE (200, 400 mg/kg) or EAF (100–400 mg/kg) did not change the anxiety level in the elevated plus maze or motor activity of the animals evaluated and open field test, respectively. A statistical trend to increase the open arm exploration was observed with CE at 800 mg/Kg. An antidepressant-like effect was detected with EAF at 400 mg/kg. Both CE (800 mg/kg) and EAF (200 and 400 mg/kg), improved memory in mice as measured by an increased latency in the step-down inhibitory avoidance test. The EAF presented higher total phenolics and tannins as compared to CE as well as it exhibited the best antioxidant activity.

Extracts of many plant species that contain a number of polyphenolic compounds have been shown to present antioxidant properties. The antioxidant activity of polyphenolics has been attributed to their redox properties, which allow them to act as reducing agents or hydrogen-atom donors. In the present study, a higher antioxidant activity was observed with EAF as compared to Vitamin C, Trolox® or CE. EAF also exhibited the higher levels of total phenolics and tannins, suggesting a close relationship between these compounds and antioxidant activity. The larger phenolics and tannins presence in the EAF may be explained by more solubility of these compounds in ethyl acetate solvent used in EAF production. Previous chemical studies have indicated the presence of the phenylpropanoid-substituted epicatechins, catiguanin A and catiguanin B along with the cinchonains Ia, Ib, Ic and Id in the barks of *Trichilia catigua* (Beltrame et al., 2006; Tang et al., 2007). These compounds exhibited potent antioxidant activity in the DPPH radical scavenging test, with IC_{50} values in the 2.3–9.4 μM range (Tang et al., 2007). In this way, antioxidant properties have been related to some of pharmacological effects of catechins and cinchonains. For example, a neuroprotective role based on antioxidant activities has been attributed to epicatechins from green tea (Weinreb et al., 2009). Uchino et al. (2002) have demonstrated that cinchocians from *Anemopaegma mirandum* reduced hepatic fibrosis in rats by suppressing oxidative stress. Thus, it is possible that both functional and antioxidant activities of EAF observed in the present work are related.

The EPM has been classically used to evaluate anxiolytic and anxiogenic effects of drugs (Lister, 1987). In the present work the

treatment of mice with diazepam, a benzodiazepine anxiolytic drug, lead to a significant increase in the open arm exploration in the EPM, without change the number of CAE, an index of locomotor activity. Although no effect on anxiety's or motor's parameters was detected after CE (200, 400 mg/kg) or EAF administration (100–400 mg/Kg), a trend in increasing the open arm exploration was observed after CE 800 mg/kg administration. Thus, it is possible that in higher doses than 800 mg/kg, the CE may present anxiolytic-like effects in mice.

In Brazil, *Trichilia catigua* preparations have been used to increase physical endurance, ameliorate performance in mental tasks and counteract stress (Mendes and Carlini, 2007). In the present study, antidepressant-like effects were detected after acute oral administration of EAF at 200 mg/kg. This effect was not shared by the animals treated with CE, in contrast with others showing antidepressant-like effects for hydroalcoholic extract of *Trichilia catigua* (Campos et al., 2005). It is possible that the solvent utilized the extract preparation have influenced the result in the FST, since in this work CE was obtained after acetone:water (7:3) extraction while in the other work hydroethanolic extract was obtained with ethanol:water (4:1) extraction. Notably, the greatest total tannins and total phenolics contents were detected in EAF, which presented antidepressant-like effect in the FST. Since psychostimulants are also shown to reduce immobility in the FST but in contrast to antidepressants they cause a marked motor stimulation, the locomotor activity of *Trichilia catigua* preparations were also evaluated in the open field test. At the same doses that EAF produce an antidepressant-like effect, it did not show significant locomotor activity augmentation. The reason for a punctual antidepressant-like effect observed with EAF at 200 mg/kg, is unclear. Complex interactions likely exist between psychoactive plant constituents of EAF to produce the observed behavioral effects and further studies are necessary to clarify this point.

The mechanistic basis of the pharmacological activity of antioxidants are multifunctional and may involve its general free radical scavenge ability (Anekonda and Reddy, 2005). Herein, animals treated with CE (200 mg/kg) or EAF (200 and 400 mg/kg) before test session presented an enhanced latency to step-down, implicating that these treatments improved memory retention. Accordingly, the positive control piracetam increased the latency to step-down. Experimental and clinical findings have associated oxidative stress and impairment of learning and memory (Cruz et al., 2003; El-Sherbiny et al., 2003). The aqueous extract of *Celastrus paniculatus* seeds, for example, was shown to have cognitive-enhancing properties paralleled with a significant decrease in malondial-dehyde (MDA), an of the reactive oxidative species, and simultaneous increase in glutathione (GHS) brain levels (Kumar and Gupta, 2002). In addition, the maintenance of normal GSH level was also reported to be important for acquisition of spatial memory in rats (Cruz et al., 2003). Thus, it is possible that the CE and EAF exerted its cognitive-enhancing activity through antioxidant activity. However, this result must be interpreted with caution and must be better investigated since memory-enhancing action may involve a combination of antioxidant, anti-inflammatory and several neurochemical transduction signals rather than the chemical antioxidant properties alone.

Pharmacological effects including antidepressant-like effects, potential antioxidant activity and positive effect on memory have been described for *Hypericum perforatum* extract (El-Sherbiny et al., 2003), an herbal antidepressant traditionally used worldwide (Barnes et al., 2001). Curiously, *Trichilia catigua* and *Hypericum perforatum* seem to share some biological properties. Besides their antioxidant activity, both extracts present antidepressant-like effects probably mediated by blocking the reuptake of dopamine rather than serotonin (Müller, 2003; Campos et al., 2005). In addition, in a passive avoidance response test on the mouse, *Hypericum perforatum* improved memory acquisition and consolidation (Klusa et al., 2001). All together, these observations suggest that *Trichilia catigua* could be useful as an antidepressant with memory enhancing properties.

In conclusion, EAF obtained from the barks of *Trichilia catigua* presented antidepressant-like effect and ameliorated memory in mice. EAF also exhibited significant *in vitro* antioxidant activity. The antioxidant activity of EAF as well as its pharmacological effects may be related to the presence of polyphenols and tannins, implicating both functional and chemical activities may be related. Further studies aimed to identify the molecules responsible for the observed pharmacological effects will be performed.

Acknowledgments

The authors would like to thank Laboratórios Catarinense for supplying the plant material. The authors acknowledge also the financial support from Conselho Nacional de Desenvolvimento Científico e tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação Araucária.

References

Amarowicz, R., Pegg, R.B., Rahimi-Moghaddam, P., Barl, B., Weil, J.A., 2004. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. Food Chemistry 84, 551–562.

Anekonda, T.S., Reddy, P.H., 2005. Can herbs provide a new generation of drugs for treating Alzheimer's disease? Brain Research Brain Research Reviews 50, 361–376.

Antunes, E., Gordo, W.M., De Oliveira, J.F., Teixeira, C.E., Hyslop, S., De Nucci, G., 2001. The relaxation of isolated rabbit *Corpus cavernosum* by the herbal medicine Catuama and its constituents. Phytotherapy Research 15, 416–421.

Barnes, J., Anderson, L.A., Phillipson, J.D., 2001. St John's wort (*Hypericum perforatum* L.): a review of its chemistry pharmacology and clinical properties. Journal of Pharmacy and Pharmacology 53, 583–600.

Barros, D.M., Mello e Souza, T., De Souza, M.M., Choi, H., Dedavid e Silva, T., Lenz, G., Medina, J.H., Izquierdo, I., 2001. LY294002, an inhibitor of phosphoinositide 3-kinase given into rat hippocampus impairs acquisition, consolidation and retrieval of memory for one-trial step-down inhibitory avoidance. Behavior Pharmacology 12, 629–634.

Beltrame, F.L., Rodrigues Filho, E., Barros, F.A.P., Cortez, D.A.G., Cass, Q.B.A., 2006. Validated higher-performance liquid chromatography method for quantification of cinchonain ib in bark and phytopharmaceuticals of *Trichilia catigua* used as catuaba. Journal of Chromatography 25, 7–263.

Brighente, I.M.C., Dias, M., Verdi, L.G., Pizzolatti, M.G., 2007. Antioxidant activity and total phenolic content of some Brazilian species. Pharmaceutical Biology 45, 156–161.

Calixto, J.B., Cabrini, D.A., 1997. Herbal medicine Catuama induces endothelium-dependent and -independent vasorelaxant action on isolated vessels from rats, guinea-pigs and rabbits. Phytotherapy Research 11, 32–38.

Campos, M.M., Fernandes, E.S., Ferreira, J., Santos, A.R., Calixto, J.B., 2005. Antidepressant-like effects of *Trichilia catigua* (Catuaba) extract: evidence for dopaminergic-mediated mechanisms. Psychopharmacology (Berlin) 182, 45–53.

Cruz, R., Almaguer, M., Bergado, W., Rosado, J.A., 2003. Glutathione in cognitive function and neurodegeneration. Reviews on Neurology 36, 877–886.

Davydov, M., Krikorian, A.D., 2000. Eleutherococcus senticosus (Rupr & Maxim.) Maxim. (Araliaceae) as an adaptogen: a closer look. Journal of Ethnopharmacology 72, 345–393.

El-Sherbiny, D.A., Khalifa, A.E., Attia, A.S., Eldenshary, Eel-D., 2003. Hypericum perforatum extract demonstrates antioxidant properties against elevated rat brain oxidative status induced by amnestic dose of scopolamine. Pharmacology Biochemistry and Behavior 76, 525–533.

Ergün, Y., Orhan, F.O., Karaaslan, M.F., 2008. Combination therapy of imipramine and melatonin: additive antidepressant effect in mouse forced swimming test. European Journal of Pharmacology 591, 159–163.

European Pharmacopoeia, 2007. Determination of Tannins in Herbal Drugs, 6 ed. European Directorate for the Quality of Medicines, pp. A286.

Klusa, V., Germane, S., Nöldner, M., Chatterjee, S.S., 2001. Hypericum extract and hyperforin: memory-enhancing properties in rodents. Pharmacopsychiatry 34, S61–S69.

Kumar, M.H., Gupta, Y.K., 2002. Antioxidant property of *Celastrus paniculatus* willd.: a possible mechanism in enhancing cognition. Phytomedicine 9, 302–311.

Lister, R.G., 1987. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology (Berlin) 92, 180–185.

Lolli, L.F., Sato, C.M., Romanini, C.V., Villas-Boas, L.B., Santos, C.A., Oliveira, R.M., 2007. Possible involvement of GABA A-benzodiazepine receptor in the anxiolytic-like effect induced by *Passiflora actinia* extracts in mice. Journal of Ethnopharmacology 111, 308–314.

Mendes, F.R., Carlini, E.A., 2007. Brazilian plants as possible adaptogens: an ethnopharmacological survey of books edited in Brazil. Journal of Ethnopharmacology 109, 493–500.

Müller, W.E., 2003. Current St John's wort research from mode of action to clinical efficacy. Pharmacological Research 47, 101–109.

Pizzolatti, M.G., Venson, A.F., Smânia, A.J., Smânia, E.F.A., Braz-Filho, R., 2002. Two epimeric flavalignans from *Trichilia catigua* (Meliaceae) with antimicrobial activity. Zeitschrift der Naturforschung 57, 483–488.

Porsolt, R.D., Bertin, A., Jalfre, M., 1977. Behavioral despair in mice: a primary screening test for antidepressants. Archives of Internal Pharmacodynamics and Therapeutics 229, 327–336.

Quintão, N.L.M., Ferreira, J., Beirith, A., Campos, M.M., Calixto, J.B., 2008. Evaluation of the effects of the herbal product Catuama® in inflammatory and neuropathic models of nociception in rats. Phytomedicine 15, 245–252.

Royce, J.R., 1977. On the construct validity of open-field measures. Psychological Bulletin 84, 1098–1106.

Tang, W., Hioki, H., Harada, K., Kubo, M., Fukuyama, Y., 2007. Antioxidant phenylpropanoid-substituted epicatechins from *Trichilia catigua*. Journal of Natural Products 70, 2010–2013.

Uchino, T., Tokunaga, H., Onodera, H., Ando, M., 2002. Effect of squalene monohydroperoxide on cytotoxicity and cytokine release in a three-dimensional human skin model and human epidermal keratinocytes. Biological and Pharmaceutical Bulletin 25, 605–610.

Verza, S.G., kreinecker, M.T., Reis, V., Henriques, A.T., Ortega, G.G., 2007. Avaliação das variáveis analíticas do método folin-ciocalteu para determinação do teor de taninos totais utilizando como modelo o extrato aquoso de folhas de Psidium guajava L. Química Nova 4, 815–820.

Viana, A.F., Maciel, I.S., Motta, E.M., Leal, P.C., Pianowski, L., Campos, M.M., Calixto, J.B., 2009. Antinociceptive activity of *Trichilia catigua* hydroalcoholic extract: new evidence on its dopaminergic effects. Evidence Based and Complementary Alternative Medicine 2011, 120820.

Weinreb, O., Amit, T., Mandel, S., Youdim, M.B., 2009. Neuroprotective molecular mechanisms of (−)-epigallocatechin-3-gallate: a reflective outcome of its antioxidant, iron chelating and neuritogenic properties. Genes and Nutrition 4, 283–296.